DETAILED ACTION

Status

Applicant's response filed 09/16/2009 is acknowledged. Claims 37, 39, 43, 44, 45, 60 and 62 are pending in the application. The previous Office action mailed 06/16/2009 indicated these claims would be allowable if presented in independent form. Upon further consideration, new grounds of rejection are set forth below. Therefore, this Office action is NON-FINAL.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 37, 39, 60 and 62 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Each of these claims recites in the first step: "forming microemulsions comprising one or more species of analyte DNA" (emphasis added). However, in the last step of claims 37 and 60 recites: "isolating using fluorescence activated cell sorting product beads which are bound to a plurality of copies of a first species of analyte DNA molecule from product beads which are bound to a plurality of copies of a second species of analyte DNA molecule". It is submitted that the last step of the claims cannot be performed if there is only "one" species of analyte DNA as alternatively recited in the first step of the claims.

A similar situation occurs for claims 39 and 62, which recite the "one or more species of analyte DNA" in the first step, while reciting in the next-to-last step: "isolating product beads which are bound to a plurality of copies of a first species of analyte DNA molecule from product beads which are bound to a plurality of copies of a second species of analyte DNA molecule". Again, this step cannot be performed in the situation where there is only "one" species of analyte DNA as alternatively recited in the first step of the claims.

One way to obviate the rejection would be to simply recite "more than one [or multiple, a plurality, etc] species of analyte DNA" instead of "one or more" in the first step of the claims.

Alternatively, for claims 37 and 60, the last step could use language such as "analyzing the product beads by fluorescence activated cell sorting, and, if multiple species of analyte DNA are present, isolating product beads which are bound to a plurality of copies of a first species of analyte DNA molecule from product beads which are bound to a plurality of copies of a second species of analyte DNA molecule". As another option, the last two steps of claims 37 and 60 could be combined to read "determining a sequence feature of at least one species of analyte DNA molecule bound to the product beads using fluorescence activated cell sorting". The claims could end there, or continue with "and, if multiple species of analyte DNA are present, isolating product beads which are bound to a plurality of copies of a first species of analyte DNA molecule from product beads which are bound to a plurality of copies of a second species of analyte DNA molecule". Any of these options would still distinguish over the

closest prior art (Leamon et al, US 7,323,305) since there would have been no reason to modify the prior art method to incorporate flow cytometry.

For claims 39 and 62, the next-to-last step could be amended to read "isolating product beads with are bound to a plurality of copies of a first species of analyte DNA molecule" followed by the last step of "amplifying the first species of analyte DNA molecule from the isolated product beads". With this language, product beads having a first species of analyte DNA are isolated, and the analyte DNA amplified therefrom, regardless of whether there was one, or more than one, species of analyte DNA as recited in the first step of the claims. The claims would still be distinguished over the method of the Leamon patent, since the Leamon method involved distributing the beads over an array of reaction chambers and performing sequencing reactions. There would have been no reason to then attempt to isolate a particular bead from the array and further amplify the nucleic acid attached thereto.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
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> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claim 44 is rejected under 35 U.S.C. 102(e) as being anticipated by Leamon et al (US 7,323,305). Based upon the declaration submitted under 37 CFR 1.131 on

03/23/2009, Applicant established reduction to practice for claim 44 prior to June 6, 2003. Therefore, this rejection can only be based on subject matter disclosed in the Leamon patent that is supported under 35 USC 112, 1st paragraph by either of Leamon's provisional applications 60/443,471 or 60/465,071. The '071 provisional application, filed 4/23/2003, provides support for the subject matter relied upon for this rejection.

With regard to claim 44, Leamon taught:

forming microemulsions comprising one or more species of analyte DNA molecules

See Leamon claim 1, step b: "delivering the fragmented nucleic acids into aqueous microreactors in a water-in-oil emulsion such that a plurality of aqueous microreactors comprise a single copy of a fragmented nucleic acid, a single bead capable of binding to the fragmented nucleic acid, and amplification reaction solution containing reagents necessary to perform nucleic acid amplification".

See provisional application 60/465,071, paragraph spanning pages 46-47:

> A second approach to amplifying and capturing both strands will be to amplify the fragment library offline in a single tube using oil and surfactant-based emulsions to encapsulate the capture beads, template and PCR reaction mix. This approach will maintain the clonality of the amplification, provide a single-tube format for second strand removal, sequencing primer annealing and the addition of signal-producing enzymes. The average size of the emulsion capsules must be optimized to maximize the number of single beads containing single strands of DNA, that can be incorporated within a single emulsion volume. An adequate volume-to-bead ratio must be maintained in order to insure a maximum number single bead capsules.

amplifying analyte DNA molecules in the microemulsions in the presence of reagent beads, wherein the reagent beads are bound to a plurality of molecules of a

primer for amplifying the analyte DNA molecules, whereby product beads are formed which are bound to a plurality of copies of one species of analyte DNA molecule

See Leamon claim 1, step c: "amplifying the fragmented nucleic acids in the microreactors to form amplified copies of said nucleic acids and binding the amplified copies to beads in the microreactors".

See Leamon, figures 35-37 and column 7, lines 14-22.

See provisional application 60/465,071, paragraph spanning pages 46-47, quoted above.

See also provisional application 60/465,071, paragraph spanning pages 43-44:

> Each bead is covalently loaded with large numbers of two oligonucleotides complementary to the 3' ends of our two universal linker sequences found on each strand of the amplified product. We will specifically capture (Figure 4) the single stranded forms of the complementary strands of an amplified DNA fragment by using these two oligonucleotides. These oligonucleotides are each targeting the 3' end of their respective stands. Both capture oligonucleotides are conjugated to the solid phase capture bead resulting in the simultaneous capture of the many copies of each strand on the same single bead in a single well.

Note that although in this paragraph, the amplification was discussed as being performed in a sealed well, Leamon's disclosure of the "second approach to amplifying and capturing both strands" (paragraph spanning pages 46-47, quoted above) clearly conveys the contemplation of performing the same amplification in an emulsion.

separating the product beads from analyte DNA molecules which are not bound to product beads

See Leamon figure 37B: "2nd strand removal".

See provisional application 60/465,071, paragraph spanning pages 46-47, quoted above: "…provide a single-tube format for second strand removal...".

determining relative or absolute amounts of product beads comprising one or more sequence features

See Leamon, claim 1, steps d and e: "...delivering the beads to an array of at least 10,000 reaction chambers...performing a sequencing reaction simultaneously on a plurality of the reaction chambers."

See provisional application 60/465,071, page 52, mid-page:

> Since we do not use robotics, but rather count on a Poisson distribution approach for bead deposition, and since we need some practical area for holding the PicoTiterPlate™, we currently only use 272K wells out of 800K wells on a 30 X 60 mm PicoTiterPlate™, to perform sequencing.

See also provisional application 60/465,071, page 2:

> The 454 Corporation has developed a massively parallel, high-throughput sequencing instrument that combines simultaneous sequencing in *hundreds of thousands* of picoliter-scale reaction wells, with high-powered bioinformatics.

As to the "determining relative or absolute amounts of product beads comprising one or more sequence features" limitation of instant claim 44, it is asserted that, by distributing the beads in at least 10,000 reaction chambers, and by simultaneously sequencing the nucleic acid fragments bound to each bead, Leamon would inherently "determine[e] relative...amounts of product beads comprising one or more sequence features". For example, if 1% of the beads on the array of reaction chambers produced a given sequence, then it would have been determined, by practicing Leamon's method, that 1% of the product beads comprised that sequence feature. Even if all 10,000 beads ended up having a different sequence, it would have been determined that 0.0001% of the beads had a given sequence.

Conclusion

Claim 43 is allowed. Claim 45is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. If the rejection under 35 USC 112, 2nd paragraph is obviated, those claims would be allowable as well.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL C. WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/
Examiner, Art Unit 1637